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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

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TITLE OF THE INVENTION (280 characters max)

COMPOSITION AND METHOD FOR INDUCING APOPTOSIS IN PROSTATE CANCER CELLS

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Respectfully submitted,

SIGNATURE: Leona G. Young   Date: April 1, 1999

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☐ Additional inventors are being named on separately numbered sheets attached hereto.

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COMPOSITION AND METHOD FOR INDUCING APOPTOSIS IN PROSTATE CANCER CELLS

FIELD OF THE INVENTION

The present invention relates to a composition comprising a mycobacterial deoxyribonucleic acid (B-DNA) and a B-DNA-mycobacterial cell wall complex, (BCC), wherein the B-DNA is complexed with and presented on the mycobacterial cell wall, such that the B-DNA and the mycobacterial DNA-bacterial cell wall complex are effective in inducing apoptosis in prostate cancer cells. More particularly, the present invention relates to a *Mycobacterium pheli* (*M. phlei*)-DNA (M-DNA) and a M-DNA *M. phlei* cell wall complex (MCC),wherein the M-DNA is complexed with and presented on the *M. phlei* cell wall, such that the MCC is effective in inducing apoptosis in prostate cancer cells.

BACKGROUND OF THE INVENTION

Cancer is an aberrant net accumulation of atypical cells, which can result from an excess of proliferation, an insufficiency of apoptosis, or a combination of the two. Apoptosis is an active cellular death process characterized by distinctive morphological changes that include condensation of nuclear chromatin, cell shrinkage, nuclear disintegration, plasma membrane blebbing, and the formation of membrane-bound apoptotic bodies (Wyllie et al. Int. Rev. Cytol. 68:251, 1980). A molecular hallmark of apoptosis is degradation of the cell's nuclear DNA into oligonucleosomal-length fragments as the result of

activation of endogenous endonucleases (Wyllie A.H. Nature 284:555, 1981).

Prostate cancer has become the most common human cancer of the internal organs and is a major cause of cancer deaths among the male population. For patients with capsule confined prostate cancer, radical prostatectomy, radiotherapy, hyperthermia, a combination of the two and brachytherapy with radiolabeled seed implants are the most commonly applied treatments. Radical prostatectomy can result in impotence and incontinence and radiotherapy is associated with PSA recurrence and brachytherapy can be used only in carefully selected patients.

For patients with extracapsular tumors and metastases, androgen ablation is the most common therapy. This includes surgical castration, bilateral orchiectomy, and chemical castration using antiandrogens, and leutinizing hormone production suppressors. Many patients refuse orchiectomy and antiandrogens and leutinizing hormone production suppressors, used together to effect total androgen blockade, result in androgen withdrawal symptoms and, also, are very expensive. Moreover, response to androgen ablation therapy is finite and lasts a median of 12 to 16 months (Crawford et al. New Eng. J. Med. 321:419, 1989).

Current chemotherapy treatments of hormone refractory (androgen independent) prostate cancer have palliation as their primary goal. However, the toxic side effects of chemotherapy treatments are debilitating and compromise the quality of life of the patient.

Therefore, there is a need for a novel therapeutic agent that inhibits proliferation of and induces apoptosis in prostate cancer cells in an animal, including a human. Moreover, such a therapeutic agent should be simple and relatively inexpensive to prepare, its activity should remain therapeutically stable over time and it should be effective at dose regimens that are associated with minimal toxicity even upon repeated administration.

SUMMARY OF THE INVENTION

The present invention satisfies the above needs by providing a composition and method comprising a mycobacterial DNA (B-DNA) and a B-DNA mycobacterial cell wall complex (BCC), wherein the B-DNA is complexed with and presented on the mycobacterial cell wall, such that the B-DNA and the BCC are effective in inhibiting proliferation of prostate cancer cells by inducing apoptosis in the prostate cancer cells. More particularly, the present invention provides a *M. phlei*-DNA (M-DNA) and a M-DNA *M. phlei* cell wall complex (MCC), wherein the M-DNA is complexed with and presented on the *M. phlei* cell wall, such that the M-DNA and the MCC are effective in inducing apoptosis in prostate cancer cells.

M-DNA and MCC are simple and relatively inexpensive to prepare, their activities are reproducible among preparations, they remain therapeutically stable over time, and they are effective at dose regimens that are associated with minimal toxicity even upon repeated administration.

To prepare MCC, *M. phlei* are grown in liquid medium and harvested. The *M. phlei* are disrupted, and the solid components of the disrupted *M. phlei* are collected by centrifugal sedimentation. The solid components are deproteinized, delipidated, and washed. M-DNA is purified from MCC or prepared directly from *M. phlei.* DNase-free reagents are used to minimize DNA degradation during preparation. of MCC and of M-DNA.

A composition comprising M-DNA or MCC and a pharmaceutically acceptable carrier is administered in a dosage sufficient to prevent, treat and eliminate prostate cancer cells in an animal, including a human. Optionally, additional therapeutic agents including, but not limited to, an anti-androgen, a chemotherapeutic agent and a steroid can be included in the M-DNA and in the MCC composition. The unexpected and surprising ability of M-DNA and of MCC to inhibit proliferation of and to induce apoptosis in prostate cancer cells addresses a long felt unfulfilled need in the medical arts and provides an important benefit for animals, including humans.

Accordingly it is an object of the present invention to provide a composition and method effective to inhibit proliferation of prostate cancer cells.

Another object of the present invention is to provide a composition and method effective to inhibit proliferation of hormone sensitive prostate cancer cells.

Another object of the present invention is to provide a composition and method effective to inhibit proliferation of hormone refractory prostate cancer cells.

Another object of the present invention is to provide a composition and method effective to induce apoptosis in prostate cancer cells.

Another object of the present invention is to provide a composition and method effective to induce apoptosis in hormone sensitive prostate cancer cells.

Another object of the present invention is to provide a composition and method effective to induce apoptosis in hormone refractory prostate cancer cells.

Another object of the present invention is to provide a composition and method effective to treat prostate cancer cells.

Another object of the present invention is to provide a composition and method effective to eliminate prostate cancer cells.

Another object of the present invention is to provide a composition and method effective to potentiate the activity of therapeutic agents in the treatment of prostate cancer cells.

Another object of the present invention is to provide a composition and method effective to potentiate the activity of anti-androgenic agents in the treatment of prostate cancer cells.

Another object of the present invention is to provide a composition and method effective to potentiate the activity of chemotherapeutic agents in the treatment of prostate cancer cells.

Another object of the present invention is to provide a composition and method effective to potentiate the activity of radiation in the treatment of prostate cancer cells.

Another object of the present invention is to provide a composition that can be prepared in large amounts.

Another object of the present invention is to provide a composition that is relatively inexpensive to prepare.

Another object of the present invention is to provide a composition that has reproducible activity among preparations.

Another object of the present invention is to provide a composition that remains stable over time.

Another object of the present invention is to provide a composition that maintains its effectiveness over time.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiment and the appended claims.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. Inhibition of proliferation of human prostate cancer cells by MCC.

FIG. 2. Inhibition of proliferation of PC3 androgen-independent human prostate cancer cells by M-DNA.

FIG. 3. Inhibition of proliferation of PC3 androgen-independent human prostate cancer cells by MCC and by DNase-treated MCC.

FIG. 4. Morphological changes in PC3 androgen independent human prostate cancer cells after treatment with MCC.

FIG. 5. Induction of DNA fragmentation in PC3 androgen independent human prostate cancer cells by MCC.

FIG. 6. Release of NuMA from PC3 androgen independent human prostate cancer cells by MCC.

DETAILED DESCRIPTION OF THE INVENTION

The present invention comprises a mycobacterial-DNA (B-DNA) and a B-DNA mycobacterial cell wall complex (BCC), wherein the bacterial DNA is complexed with and presented on the bacterial cell wall, such that the B-DNA and the BCC are effective in inhibiting

proliferation of and inducing apoptosis in prostate cancer cells in an animal. More particularly, the present invention relates to a *M. phlei*-DNA (M-DNA) and a M-DNA *M. phlei* cell wall complex (MCC), wherein the mycobacterial DNA is complexed with and presented on the mycobacterial cell wall, such that the M-DNA and the MCC are effective in inhibiting proliferation of and inducing apoptosis in prostate cancer cells in an animal, including a human.

Many bacterial species can be used to practice the present invention including, but not limited to, *Coryneform* species, *Corynebacterium* species, *Rhodococcus* species, *Eubacterium* species, *Bordetella* species, *Escherichia* species, *Listeria* species, *Nocardia* species and *Mycobacterium* species. Preferably, a *Mycobacterium* species is used including, but not limited to, *M. smegmatis, M. fortuitum, M. kansaasii, M. tuberculosis, M. bovis, M. vaccae, M. avium* and *M. phlei*. Most preferably, the *Mycobacterium* species *M. phlei* is used.

Methods to increase the therapeutic activity of the M-DNA and of the MCC composition of the present invention include, but are not limited to, chemically supplementing or biotechnologically amplifying stimulatory sequences or confirmations of DNA derived from the same or different bacterial species and complexing the M-DNA and the MCC to natural or synthetic carriers. . The M-DNA and the MCC can be supplemented with naturally occuring or chemically-synthesized nucleic acids.

Optionally, therapeutic agents including, but not limited to, anti-androgenic drugs, chemotherapeutic drugs, steroidal drugs and immunological agents can be included in the M-DNA and the MCC composition of the present invention.

Anti-androgenic drugs include, but are not limited to, flutamide, bicalutamide, nilutamide, megestrol acetate, adrenocorticotropic hormone secretion inhibitors, ketoconazole, estrogens, anti-estrogens and LHRH production suppressors.

Chemotherapeutic drugs include, but are not limited to, anti-metabolites, DNA damaging, microtubule destabilizing, microtubule stabilizing, actin depolymerizing, growth inhibiting, topoisomerase inhibiting, HMG-CoA inhibiting, purine inhibiting,

pyrimidine inhibiting, metaloproteinase inhibiting, cdK inhibiting, angiogenesis inhibiting, differentiation and immunotherapeutic drugs. These agents include, but are not limited to, anthracycline antibiotics such as doxorubicin and mitoxantrone, estramustine, vinblastine, paclitaxel, etoposide, cyclophosphamide, cisplatin, carboplatin and combinations of the above with or without the addition of steroid drugs.

Immunological agents include, but are not limited to, cytokines, chemokines, interferons, interleukins, or polyclonal or monoclonal antibodies.

The M-DNA composition and the MCC composition and the optional therapeutic agent can be administered simultaneously or sequentially on the same or different schedules..

Compositions comprising M-DNA and MCC and a pharmaceutically acceptable carrier are prepared by uniformly and intimately bringing into association the M-DNA and the MCC with liquid carriers, with solid carriers, or with both. Liquid carriers include, but are not limited to, aqueous carriers, non-aqueous carriers or both. Solid carriers include, but are not limited to, biological carriers, chemical carriers or both.

Among its pharmaceutically acceptable carriers, the M-DNA and the MCC, or the M-DNA and the MCC and a therapeutic agent, can be administered in aqueous suspension, oil emulsion, water in oil emulsion, water-in-oil-in-water emulsion, liposomes, microparticles, site-specific emulsions, long-residence emulsions, sticky-emulsions, microemulsions, nanoemulsions, microspheres, nanospheres, nanoparticles, minipumps, and with various natural or synthetic polymers that allow for sustained release of the composition.

Further, the M-DNA and the MCC, or the M-DNA and the MCC and a therapeutic agent, can be used with any one, all, or any combination of excipients regardless of the carrier used to present the composition to the responding cells. These include, but are not limited to, anti-oxidants, buffers, and bacteriostats, and may include suspending agents and thickening agents.

Preferably, the M-DNA and the MCC are administered as an aqueous suspension. The composition is suspended in a

pharmaceutically acceptable carrier such as, but not limited to, DNase-free water, saline or phosphate buffered saline (PBS) and is submicronized by sonication or microfluidization. Optionally, the submicronized mixture is homogenized by microfluidization. For example, lyophilized M-DNA or MCC are suspended in sterile water and sonicated at 20% output for 5 minutes (Model W-385 Sonicator, Heat Systems-Ultrasonics Inc). The sonicated composition is homogenized by microfluidization at 15,000-30,000 psi for one flow-through (Model M-110Y; Microfluidics, Newton, MA). The mixture is either aseptically processed or terminally sterilized. Optionally a therapeutic agent can be added to the M-DNA or MCC composition during submicronization homogenization or before or after sterilization.

For administration in a non-aqueous carrier, the M-DNA and the MCC are emulsified with a mineral oil or with a neutral oil such as, but not limited to, a diglyceride, a triglyceride, a phospholipid, a lipid, an oil and mixtures thereof, wherein the oil contains an appropriate mix of polyunsaturated and saturated fatty acids. Examples include, but are not limited to, soybean oil, canola oil, palm oil, olive oil and myglyol, wherein the number of fatty acid carbons is between 12 and 22 and wherein the fatty acids can be saturated or unsaturated. Optionally, charged lipid or phospholipid can be suspended in the neutral oil. For example, DNase free phosphatidylcholine is added to DNase free triglyceride soybean oil at a ratio of 1 gram of phospholipid to 20 ml of triglyceride and is dissolved by gentle heating at 50°-60° C. Several grams of MCC are added to a dry autoclaved container and the phospholipid-triglyceride solution is added at a concentration of 20 ml per 1 gram of MCC. The suspension is incubated for 60 min. at 20° C and is then mixed with DNase-free PBS in the ratio of 20 ml MCC suspension per liter of PBS. The mixture is emulsified by sonication at 20% output for 5 minutes (Model W-385 Sonicator, Heat Systems-Ultrasonics Inc.). Optionally, the emulsified mixture is homogenized by microfluidization at 15,000-30,000 psi for one flow-through (Model M-110Y; Microfluidics). The MCC emulsion is transferred to an autoclaved, capped bottle for storage at 4° C. Optionally a therapeutic

agent can be added to the M-DNA or the MCC composition during incubation, emulsification or homogenization..

The M-DNA and the MCC, or the M-DNA and the MCC and a therapeutic agent, are administered in an amount effective to induce a therapeutic response in cancer cells. The dosage of the M-DNA and the MCC, or of the M-DNA and the MCC and a therapeutic agent, administered will depend on the stage of the prostate cancer being treated, the organs to which it may have metastasized, the particular formulation, and other clinical factors such as the size, weight and condition of the recipient and route of administration.

Preferably, the amount of M-DNA and of MCC administered is from about 0.00001 mg/kg to about 200 mg/kg per dose, more preferably from about 0.0001 mg/kg to about 100 mg/kg per dose, and most preferably from about 0.001 mg/kg to about 50 mg/kg per dose.

Preferably, the M-DNA content of MCC is between about 0.001 mg/100 mg dry MCC and about 90 mg/100 mg dry MCC, more preferably between about 0.01 mg/100 mg dry MCC and about 40 mg/100 mg dry MCC, most preferably between about 0.1 mg/100 mg dry MCC and about 30 mg/100 mg dry MCC. -Unexpectedly, we found that at least about 3 to 4% of the dry weight of MCC is extractable M-DNA.

Routes of administration include, but are not limited to, oral, topical, subcutaneous, intra-muscular, intra-peritoneal, intra-venous, intra-dermal, intra-thecal, intra-lesional, intra-tumoral, intra-bladder, intra-vaginal, intra-ocular, intra-rectal, intra-pulmonary, intra-spinal, transdermal, subdermal, placement within cavities of the body, nasal inhalation, pulmonary inhalation, impression into skin and electrocorporation.

Depending on the route of administration, the volume per dose is preferably about 0.001 ml to about 100 ml per dose, more preferably about 0.01 ml to about 50 ml per dose and most preferably about 0.1 ml to about 30 ml per dose. The M-DNA and the MCC, or the M-DNA and the MCC and a therapeutic agent, can be administered in a single dose treatment or in multiple dose treatments on a schedule and

over a period of time appropriate to the stage of the prostate cancer being treated, the organs to which it has metastasized, the condition of the recipient and the route of administration.

The following examples will serve to further illustrate the present invention without, at the same time, however, constituting any limitation thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

EXAMPLE 1

Preparation of MCC from M. phlei and purification of M. phlei DNA

MCC was prepared from *M. phlei* (*M. phlei*) (strain 110), M-DNA was purified from *M. phlei* (M-DNA) as described in International Patent Application No. PCT/CA98/00744, which is included by reference herein. All reagents were selected to enhance conservation of the DNA. Unless stated otherwise, MCC, and M-DNA were resuspended in DNase-free water or in a pharmaceutically acceptable DNase-free buffer and emulsified by sonication. MCC and M-DNA did not contain endotoxins as determined using a Limulus amebocyte lysate QCL-1000 kit (BioWhittaker, Walkersville, MD).

EXAMPLE 2

Preparation of bacterial-DNA-bacterial cell wall complex and of bacterial DNA from other bacterial species

Bacterial DNA-bacterial cell wall complex and bacterial-DNA are prepared from *M. smegmatis, M. fortuitous, Nocardia rubra, Nocardia asteroides, Cornybacterium parvum, M. kansaasii, M tuberculosis* and *M. bovis* as in Example 1.

EXAMPLE 3

Cells and reagents

PC3, DU-145 androgen independent and LNCaP androgen dependent prostate cancer cells were obtained from the ATCC (#CRL-1435, HTB-81 and CRL-1740, respectively) and primary human prostate epithelial cells (PrEc) from Clonetics (#CC-2555). All cell lines were cultured by standard techniques. PC3, DU-145, LNCaP and PrEc cells were seeded at 3 X 10^5 cells/ml of growth medium in 6-well flat bottom microplates and allowed to grow for 24 h at 37° C, 5% CO_2. At 24 h, medium was replaced with medium containing MCC or M-DNA

EXAMPLE 4

DNase I treatment of MCC

Treatment of MCC with DNase I was carried out as described in International Patent Application No. PCT/CA98/00744.

EXAMPLE 5

Inhibition of prostate cancer cell proliferation by MCC

Cell proliferation was determined using dimethylthiazol-diphenyltetrazolium bromide (MTT) reduction (Mosman et al. J. of Immunol. Meth. 65:55, 1983).

PC3, DU-145 and LNCaP, human prostate cancer cell lines, and PrEc, a primary human prostate epithelial cell line, were incubated with 0.01, 0.10, 1, 10 and 100 μg/ml of MCC. After 48 h incubation, 100 μl of MTT in 5mg/ml phosphate buffered saline was added to each well and incubation was continued for 4 h. Medium was then aspirated from each well, 1 ml of acidified isopropyl alcohol was added, and reduced MTT was solubilized by mixing. The absorbency of the reaction product was determined at 570 nm.

As shown in Fig. 1, MCC at 1, 10 and 100 μg/ml inhibited proliferation of LNCaP androgen dependent prostate cancer cells and of PC3 and DU-145 androgen independent prostate cancer cells, but not of PrEc normal prostate cells.

These data show that MCC inhibits proliferation of both androgen independent and androgen dependent prostate cancer cells in

the absence of immune effector cells. MCC does not inhibit proliferation of normal prostate cells.

EXAMPLE 6

Inhibition of cell proliferation by M-DNA

PC3 androgen dependent prostate cancer cells were incubated with 10 μg/ml and with 100 μg/ml of M-DNA. Cell proliferation was determined as in Example 5.

As shown in Fig. 2, at 10 μg/ml and at 100 μg/ml M-DNA inhibited proliferation of PC3 androgen dependent prostate cancer cells about 30%.

These data show that M-DNA inhibits cell proliferation of PC3 androgen dependent prostate cancer in the absence of immune effector cells.

EXAMPLE 7

Inhibition of cell proliferation by MCC and by DNase I treated MCC

PC3 androgen independent prostate cancer cells were incubated with 1 Unit/ml DNase I with 1 μg/ml MCC and with 1 μg/ml DNase I treated MCC.

As shown in Fig. 3, DNase I inhibited proliferation PC3 androgen independent prostate cancer cells by about 5%, MCC by about 50% and DNase I treated MCC by about 30%.

These data show that MCC inhibits proliferation of PC3 androgen independent prostate cancer cells in the absence of immune effector cells. Further, these data show that treatment of MCC with DNase I, which degrades both single and double stranded DNA, reduces the anti-proliferative effect of MCC.

EXAMPLE 8

Induction of apoptosis as indicated by morphological changes

Morphological changes that include condensation of nuclear chromatin, cell shrinkage, nuclear disintegration, plasma membrane blebbing and the formation of membrane-bound bodies are

indicative of cell death by apoptosis (Wyllie et al. Int. Rev. Cytol. 68:251,1980).

PC3 androgen independent prostate cancer cells were incubated with 0 μg/ml MCC and with 200 μg/ml of MCC for 48 h. Images were collected on a light microscope with a 40 x 2.5 NA Apochromat objective.

As shown in Figure 4, cells incubated with 0 μg/ml MCC exhibited normal morphology, whereas cells incubated with 200 μg/ml MCC exhibited striking morphological changes indicative of cell death by apoptosis.

These date show that MCC induces apoptosis in PC3 androgen independent prostate cancer cells in the absence of immune effector cells.

EXAMPLE 9

Induction of apoptosis as indicated by DNA fragmentation

Fragmentation of cellular DNA into nucleosome-sized fragments by the activation of endogenous endonucleases is characteristic of cells undergoing apoptosis (Wyllie A.H. Nature 284:555, 1981; Newell et al. Nature 357:286, 1990).

During routine monolayer culture of PC3 cells, a considerable number of cells were observed to detach from the plastic surface of the tissue culture wells and to float in the medium (Palayoor et al., Radiation Res. 148:105, 1997). The proportion of detached cells increased after 48 h of MCC treatment. Therefore, DNA fragmentation was analyzed both in detached and in attached PC3 cells (Smith et al. Nature 337:795, 1989).

Detached cells were collected and centrifuged at 350 X G for 5 min. The pellet of detached cells and the remaining attached cells were lysed in 50 μl of a buffer containing 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.5 % (w/v) sodium lauryl sarcosinate (SDS) and 0.5 mg/ml proteinase K at 55°C for 1 h. Ten μl of 0.5 mg/ml RNase A was added to each sample and the incubation was continued for 1 h. Samples were heated to 65°C and 10 μl of 10 mM EDTA (pH 8.0) containing 1 % (w/v) low-gelling-temperature agarose, 0.25 % (w/v) bromophenol blue,

40 % (w/v) sucrose was mixed with each sample. The samples were electrophoresed at 100 V for 3 h in a 2 % agarose gel containing Tris-borate/EDTA buffer (TBE). The DNA was visualized under UV transillumination using ethidium bromide staining

PC3 androgen independent prostate cancer cells were incubated for 48 h with 0 μg/ml MCC and with 100μg/ml MCC. Detached cells treated with 100 μg/ml MCC showed significant DNA fragmentation (Fig. 5, lanes 3 and 4). Cells treated with 0 μg/ml MCC (Fig. 5, lane 1) and attached cells treated with 100μg/ml MCC showed no fragmentation (Fig. 5, lane 2).

These data show that MCC induces apoptosis, as indicated by DNA fragmentation, in detached PC3 androgen independent prostate cancer cells in the absence of immune effector cells.

EXAMPLE 10

Induction of apoptosis as indicated by release of NuMA protein

The induction of apoptosis may also be demonstrated by the solubilization and release of nuclear matrix (NuMA) protein. PC3 androgen independent prostate cancer cells were incubated for 48 h with 0 μg/ml MCC and with 200μg/ml MCC. Medium was drawn off, centrifuged at 10 000 X G to remove MCC and the supernatant was frozen at -20° C until assayed for NuMA (Miller et al. Biotech. 15:1042, 1993).

As shown in Figure 7, PC3 androgen independent prostate cells treated with 200 μg/ml MCC released 20% more NuMA proteins into the medium than did cells treated with 0 μg/ml MCC.

These date show that MCC induces apoptosis, as indicated by NuMA release, in PC3 androgen independent prostate cancer cells in the absence of immune effector cells.

EXAMPLE 11

Effects of MCC, M-DNA and DNase I treated MCC on PC3 tumors in mice

PC3 androgen independent prostate cancer cells are implanted subcutaneously into 40 male nude BALB/c mice and allowed

to grow until palpable (0.1 to 0.5 cm in diameter). The mice are divided into 4 groups and tumor mass is measured in each mouse. The 10 mice in Group 1 each receive saline on day 0. The 10 mice in Group 2 each receive saline containing MCC on day 0. The 10 mice in Group 3 each receive saline containing M-DNA. The 10 mice in Group 4 each receive DNase I treated MCC. After 4 weeks of treatment, the mice are sacrificed and the tumor mass and number of metastases are determined. The mice in Group 2 and in Group 3 have less tumor mass and fewer metastases than the mice in Group 1 and in Group 4.

EXAMPLE 12

Effects of MCC and of M-DNA alone and in combination with estramustine and etoposide on PC3 tumors in mice

PC3 androgen independent prostate cancer cells are implanted subcutaneously into 60 male nude BALB/c mice and allowed to grow until palpable (0.1 to 0.5 cm in diameter). The mice are divided into 6 groups and tumor mass is measured in each mouse. The 10 mice in Group 1 receive saline. The 10 mice in Group 2 receive MCC in saline. The 10 mice in Group 3 receive M-DNA in saline. The 10 mice in Group 4 receive estramustine and etoposide in saline. The 10 mice in Group 5 receive MCC in combination with estramustine and etoposide in saline. The 10 mice in Group 6 receive M-DNA in combination with estramustine and etoposide in saline. After 4 weeks of treatment, the mice are sacrificed and the tumor mass and number of metastases are determined. The mice in Group 1 have the most tumor mass. The mice in Group 4 have less tumor mass than the mice in Group 1. The mice in Group 2 and Group 3 have less tumor mass than the mice in Group 4. The mice in Group 5 and in Group 6 have the least tumor mass.

EXAMPLE 13

Effects of MCC, M-DNA and DNase I treated MCC on LNCaP tumors in mice

LNCaP androgen dependent prostate cancer cells are implanted subcutaneously into 40 male nude BALB/c mice and allowed to grow until palpable (0.1 to 0.5 cm in diameter). The mice are divided

into 4 groups and tumor mass is measured in each mouse. The 10 mice in Group 1 each receive saline on day 0. The 10 mice in Group 2 each receive saline containing MCC on day 0. The 10 mice in Group 3 each receive saline containing M-DNA. The 10 mice in Group 4 each receive DNase I treated MCC. After 4 weeks of treatment, the mice are sacrificed and the tumor mass and number of metastases are determined. The mice in Group 2 and in Group 3 have less tumor mass and fewer metastases than the mice in Group 1 and in Group 4

EXAMPLE 14

Effects of MCC and of M-DNA alone and in combination with flutamide on LNCaP tumors in mice

LNCaP androgen dependent prostate cancer cells are implanted subcutaneously into 60 male nude BALB/c mice and allowed to grow until palpable (0.1 to 0.5 cm in diameter). The mice are divided into 6 groups and tumor mass is measured in each mouse. The 10 mice in Group 1 receive saline. The 10 mice in Group 2 receive MCC in saline. The 10 mice in Group 3 receive M-DNA in saline. The 10 mice in Group 4 receive flutamide in saline. The 10 mice in Group 5 receive MCC in combination with flutamide in saline. The 10 mice in Group 6 receive M-DNA in combination with flutamide in saline. After 4 weeks of treatment, the mice are sacrificed and the tumor mass and number of metastases are determined. The mice in Group 1 have the most tumor mass. The mice in Group 4 have less tumor mass than the mice in Group 1. The mice in Group 2 and Group 3 have less tumor mass than the mice in Group 4. The mice in Group 5 and in Group 6 have the least tumor mass.

It should be understood, of course, that the foregoing relates only to a preferred embodiment of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

We claim:

1. A composition comprising, a mycobacterial deoxyribonucleic acid (B-DNA), a first pharmaceutically acceptable carrier and a second pharmaceutically acceptable carrier, wherein the B-DNA is effective in inducing a response in prostate cancer cells in an animal having prostate cancer.

2. The composition of claim 1, wherein the mycobacterial DNA is *M. phlei-DNA* (M-DNA).

3. The composition of claim 1, wherein the first pharmaceutically acceptable carrier is deproteinized, delipidated *M. phlei* cell wall.

4. The composition of claim 1, wherein the response is inhibition of proliferation of prostate cancer cells.

5. The composition of claim 1, wherein the response is induction of apoptosis in prostate cancer cells.

6. The composition of claim 1, further comprising a therapeutic agent.

7. A method, comprising administering to an animal a composition comprising mycobacterial deoxyribonucleic acid (B-DNA), a first pharmaceutically acceptable carrier and a second pharmaceutically acceptable carrier, wherein the B-DNA is administered in an amount effective to induce a response in prostate cancer cells of an animal having prostate cancer.

8. The method of claim 7, wherein the B-DNA is *M. phlei*-DNA (M-DNA).

9. The method of claim 7, wherein the first pharmaceutically acceptable carrier is deproteinized, delipidated *M. phlei* cell wall.

10. The composition of claim 7, wherein the response is inhibition of proliferation of prostate cancer cells.

11. The method of claim 7, further comprising a therapeutic agent.

12. A composition, comprising
a. *M. phlei*-DNA (M-DNA);
b. deproteinized, delipidated *M. phlei* cell wall, wherein the M-DNA is preserved and complexed on the *M. phlei* cell wall (MCC); and,
c. a pharmaceutically acceptable carrier, wherein the MCC is effective in inducing a response in prostate cancer cells of an animal having prostate cancer
.

13. The composition of claim 12, wherein the response in inhibition of proliferation of prostate cancer cells.

14. The composition of claim 12, wherein the response is induction of apoptosis in prostate cancer cells.

15. The composition of claim 12, further comprising a therapeutic agent.

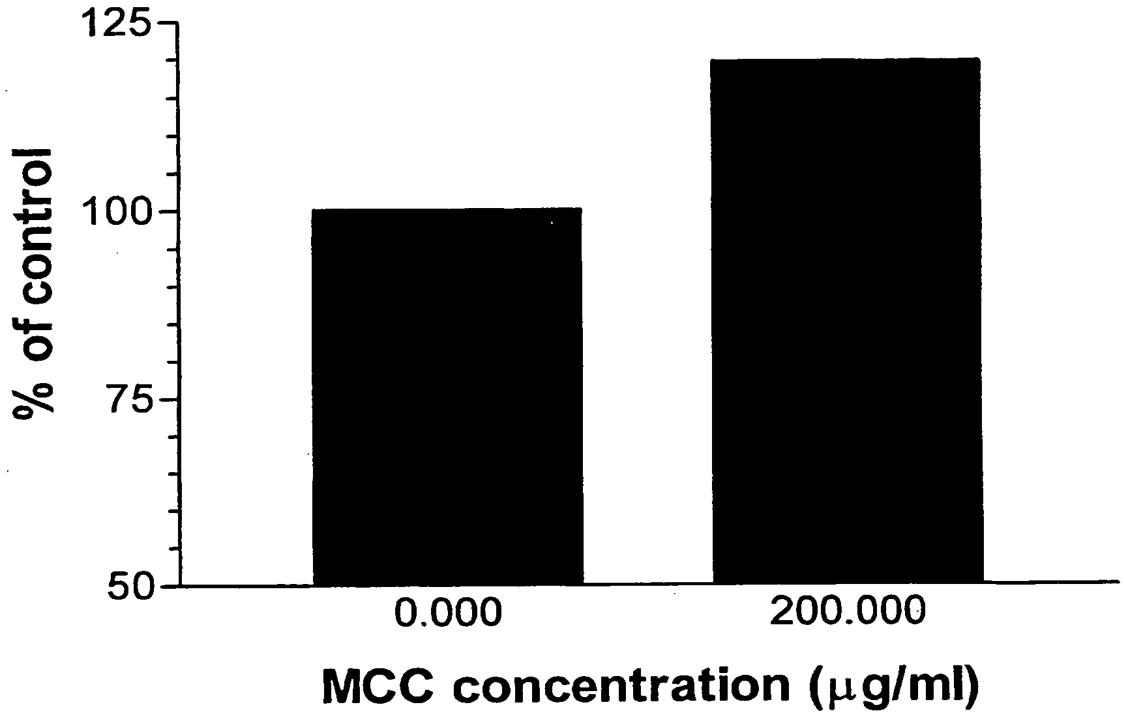
16. A method, comprising administering to an animal a composition, comprising:
a. *M. phlei*-DNA (M-DNA);
b. deproteinized, delipidated *M. phlei* cell wall, wherein the M-DNA is preserved and complexed on the *M. phlei* cell wall (MCC); and,
c. a pharmaceutically acceptable carrier,
wherein the MCC is administered in an amount effective to induce a response in prostate cancer cells in an animal having prostate cancer.

17. The method of claim 16, wherein the response in inhibition of proliferation of prostate cancer cells.

18. The method of claim 16, wherein the response is induction of apoptosis in prostate cancer cells.

19. The method of claim 16, further comprising a therapeutic agent.

Release of NuMA by PC3 human prostate cancer cells after MCC treatment



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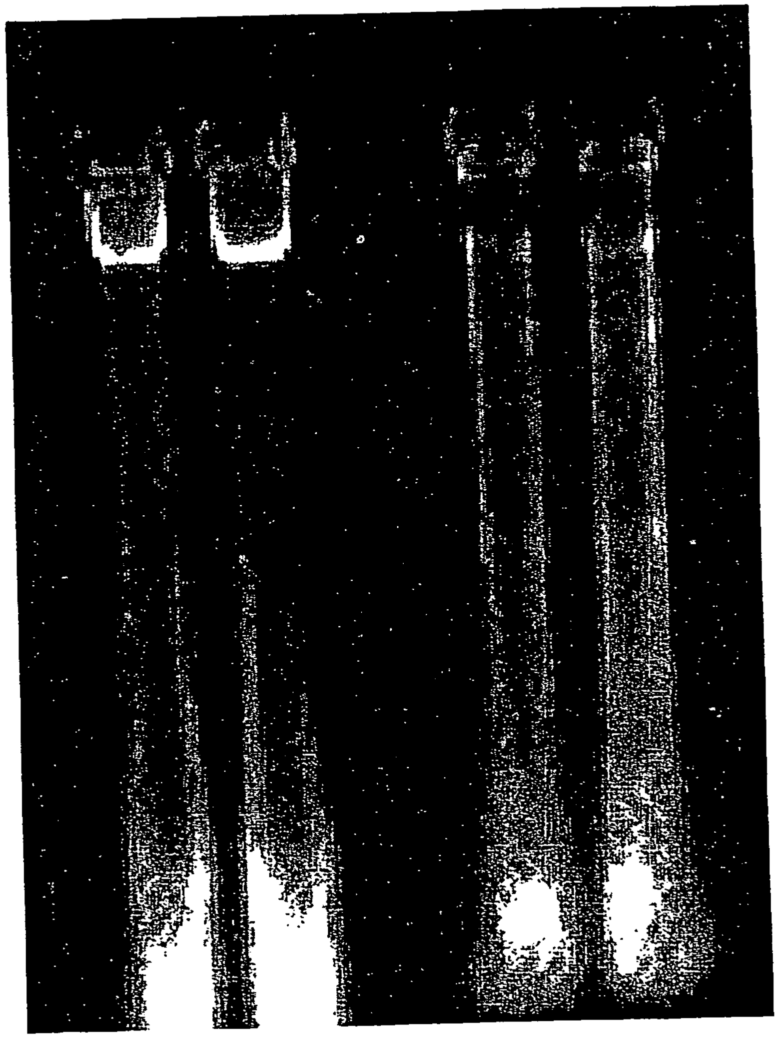
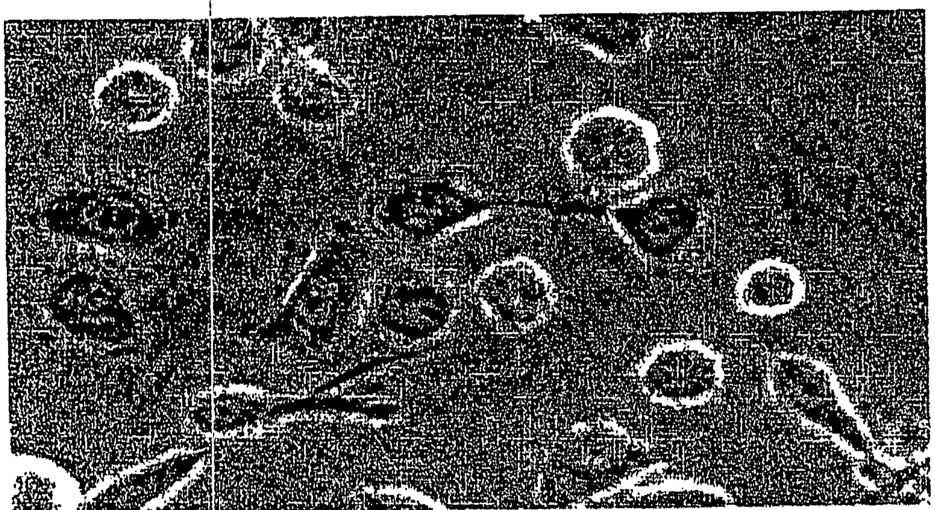


ABSTRACT

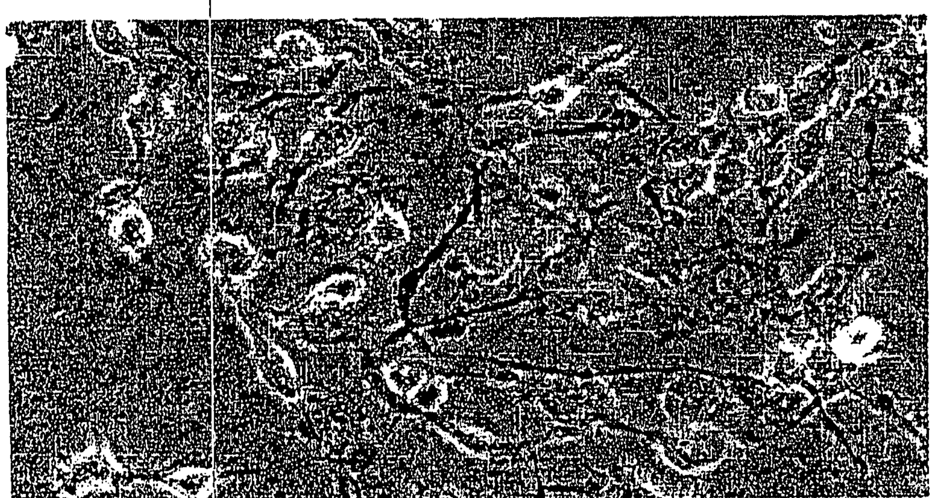
COMPOSITION AND METHOD FOR INDUCING APOPTOSIS IN PROSTATE CANCER CELLS

The present invention relates to a composition comprising a mycobacterial deoxyribonucleic acid (B-DNA) and to a B-DNA mycobacterial cell wall complex (BCC), wherein the B-DNA is complexed with and presented on mycobacterial cell wall, such that the B-DNA and the B-DNA-bacterial cell wall complex are effective in inducing apoptosis in prostate cancer cells. More particularly, the present invention relates to a *Mycobacterium phlei* (*M. phlei*)-DNA (M-DNA) and to a M-DNA *M. phlei* cell wall complex (MCC), wherein the M-DNA is complexed with and presented on the *M. phlei* cell wall, such that the MCC is effective in inducing apoptosis in prostate cancer cells.

Attorney Docket 02811-0160P

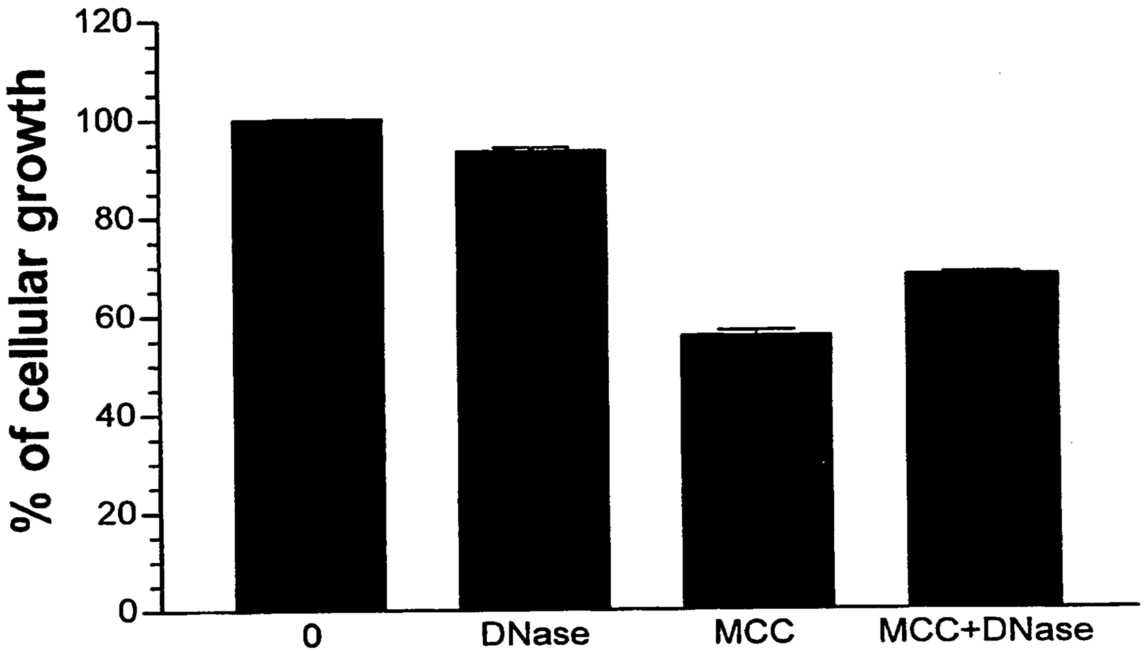


PC-3 untreated

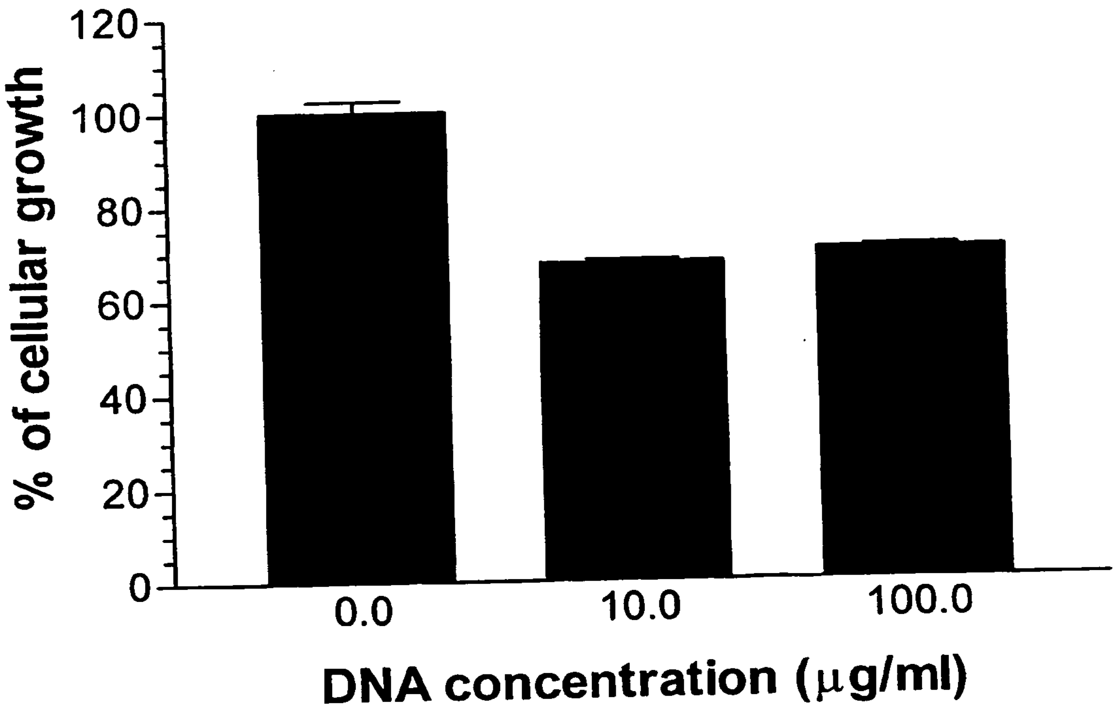


PC-3 + MCC
200 μg/ml

Inhibition of PC3 cellular proliferation by MCC: effect of DNA



Inhibition of PC3 cellular proliferation by *M. phlei*-derived DNA



Inhibition of human prostate cancer cell lines proliferation by MCC